
Hydrolytic enzyme levels in malted cereals

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Abstract: Six grain samples namely barley, maize, millet, rice, sorghum and wheat were tested for enzyme levels (amylase, protease and lipase) in order to assess their malting characteristics, using barley as a standard. The levels of the enzymes were estimated in the whole dry grains, 24h steeped, 24h sprouted and 48h sprouted grains. A combined statistical analysis of linear regression and analysis of variance were used to test for enzyme interaction, similarities and interspecific relationship between the cereals. The estimated interspecific relationship between sorghum and barley was 84% based on the three enzymes, while millet had 75%. All the sprouted cereals with the exception of the standard barley had high lipase levels. This possibly suggests certain limitations as regards to production of off-flavours. Malt prepared from maize, sorghum, rice and millet had comparable protease levels with barley ($P>0.05$). The malts prepared from maize, millet, rice, sorghum and wheat had comparatively low alpha-amylase levels when compared with the standard barley. Multiple linear regressions showed amylase was dependent on lipase and protease. The relationship was not affected by either soaking (steeping) or sprouting of the grains. The implications of these findings suggest that a combination of these cereals might give good malt for food applications.

Keywords: Amylase, Lipase, Protease, Malting, Cereals

1. Introduction

The main objective of malting is to promote synthesis of hydrolytic enzymes which will solubilize large macromolecules into low molecular weight compounds of desired characteristics. Malting is the limited germination of cereal grains or, occasionally, the seeds of pulses (peas and beans), under controlled conditions with the primary objective of promoting the development of hydrolytic enzymes [1-2]. The malting process involves germination and growth resulting in the development of enzymes which degrade the reserved food materials in the endosperm of the grain. Malting induces important beneficial biochemical changes in sorghum grains especially. Indeed, soaking/steeping generates grain softening and increases water availability [3]. The enzymes produced during germination lead to the hydrolysis of starch and proteins with release of sugars and amino acids directly available.

Several types of cereals have been used for malting. The malting potentials of different cereals have been studied from time over the time [4]. Malts from wheat, sorghum and maize have been studied with respect to their use in food formulation and also as brewing adjuncts [5]. Malting of barley is a recognized process associated with beer

production. The conversion of barley into beer represents mankind's oldest and most complex example of applied enzymology. Indeed, historically some of the most significant advances in enzymology have been linked to the world of brewing. Analysis of invertase in determining the factors that a bearing on the quality of beer, brewers have learned not only how the endogenous enzymes contribute to issues such as fermentability, filterability, foam, clarity, but also flavor [6].

However, the selection of any grain for good malting characteristics is based on the criteria that it will be suitable for processing and would yield the desired flavour qualities or characteristics. There are different physical and chemical criteria used in defining high and low grade malt [7]. A few of such tests include; germination energy, embryo growth, endosperm sedimentation score, enzyme potentials and protein content. Proteolytic enzymes improve amino acid availability, particularly lysine, methionine and tryptophan that are lacking in cereals.

This paper reports the use of a combined assessment of some hydrolytic enzymes (amylase, protease and lipase) for predicting the malting characteristics of some cereals grown in Nigeria especially north eastern region. Six types of cereals namely barley, maize, rice; sorghum and wheat were

screened for hydrolytic enzymes using barley as a reference standard.

2. Materials and Methods

Freshly harvested seeds of the six cereals namely; barley (*Hordeum spp.* Quite 33 var.), maize (*Zea mays*, Tzesr-W var.), millet (*Penisetum spp.* Ex-borno var.), rice (*Oriza sativa*, dikwa var.), Sorghum (*Sorghum bicolor*, chakalari var.) and wheat (*Triticum spp.* Siette ceros var.) were obtained from Lake Chad Research Institute, Maiduguri, Nigeria.

2.1. Malting

The seeds were steeped in distilled water at 25°C for 24 hours and then transferred to moistened layer cotton cheese cloth and thereafter allowed to germinate in a dark cupboard at room temperature 25°C for 48 hours. The sprouted seeds were devegetated, dried at 50°C in an air conventional oven and stored in air-tight containers at 40°C.

2.2. Amylase

The activity of amylase was determined according to the method of [8], based on starch-iodine colour changes. One unit of amylase activity was defined as $A_0 - A_t / A_0 \times 100$, where A_0 and A_t were the absorbance of the iodine-starch complex at zero time and after 60 minutes of hydrolysis. Specific activity of amylase was defined as units/mg protein/hour.

2.3. Lipase

The lipase activity was determined by the method of [9]. This method estimates the fatty acid produced by the hydrolysis of an olive oil emulsion on which the lipase extract from the cereals had hydrolyzed under optimum conditions of pH and temperature. One unit of lipase activity was defined as increase in 0.1 ml units of NaOH titre value over the blank assay.

2.4. Protease

Protease level was assayed by the method described by [10]. The free amino acids formed after digestion of a casein substrate was estimated by formol titration. Protease activity was calculated from the equation $(X - Y) \times 100$, where X is the titre value after proteolysis, and Y is the blank titre value equivalent to the protease free assay.

2.5. Interspecific Relationships

The statistical method described by [11] was used. The relationship were estimated as mean deviations from the standard barley values and expressed as percentages.

3. Results and Discussion

The results obtained generally showed a wide variations in the various enzyme levels for the different cereals. In general, steeping treatment has been reported to increase enzyme

activities. Reported [12] significant increase in diastatic activity when sorghum grains were hydrated for longer periods (40 hours) than 24 and 16 hours. When compared with barley, lower amylase levels were observed in maize, millet, rice, sorghum and wheat after 48 hours of sprouting. In contrast, the levels of protease were comparable to barley ($P > 0.05$) with the exception of wheat which notably had the highest value. Lipase in the sprouted maize, millet, sorghum and wheat were comparatively higher than that of barley, while rice had a lower value than the reference standard.

In the malting process, the hard endosperm is enzymically converted into friable malt and alpha amylase plays a crucial role in this respect. Malting of rice increased the quantity of hydrolytic enzymes (amylase, protease) present in them resulting in products that are more nutritious than those *kunun-zaki* produced without the addition of malted rice [13]. Since starch is the major constituent of the seed, it is expedient that amylase levels could serve as an index for predicting the desired characteristics of good malt. The hydrolytic enzyme (amylase) in the malted cereal aids in digesting the thick slurry thereby converting the complex carbohydrates in the cereal to simple sugars [12]. In line with this, the relationships between the enzymes were statistically investigated by a multiple linear regression, and amylase was found to be dependent on lipase and protease as shown in the equation:

$$Y_{\text{amylase}} = 0.97X_{\text{lipase}} - 0.05Z_{\text{protease}} - 111.85$$

From the equation, it appears that lipase levels of the cereals is more valuable than protease in predicting the α -amylase and hence the friability (soft-texture or ease of crumble ness) of the malt. Structural analysis of most cereals showed that starch granules are enveloped within a lipid vesicle and such lipid barriers can reduce the susceptibility of starch granules to amylolytic digestion [14]. Therefore, high lipase levels should improve the amylolytic conversion of the endosperm starch. Differences in lipase levels of the cereals are possibly due to the site of production of this enzyme during germination. According to [15], sorghum differs from barley with respect to lipase in that this enzyme is only limited to the aleurone layer in barley, while it is produced in both the aleurone and embryo in sorghum. Maize which had the highest levels of lipase in the study may be similar to sorghum.

When lipase and protease were considered again individually in relation to amylase using the simple regression

$$Y_{\text{amylase}} = 0.12Z_{\text{protease}} + 123.84 \quad (r=0.15)$$

$$Y_{\text{amylase}} = 1.91X_{\text{lipase}} - 288.29 \quad (r=0.65)$$

In consonance with the multiple regressions, the two equations together suggest that lipase is a stronger limiting factor than protease when predicting the friable quality of the malt. Higher lipase activity in cereals may render the malt readily susceptible to lipid peroxidation since the major fatty acids are polyunsaturated derivatives and this lead to the

production of off-flavour. Therefore it may be advisable to add synthetic food antioxidants to preserve the malt, provided they will not affect the quality of the final product for which the malt is intended.

The interspecific relationship between the cereals based on the three enzymes showed that sorghum has 84.21% relationship with barley; closely followed by millet (75%). This observation is in line with the findings of [16] who reported that sorghum had better malting characteristics than other cereals such as maize, millet, or rice. They also found that barley and sorghum exhibited similarities in nitrogen content, protease activity, diastatic activity, extract yield and tannin content.

The basic significance of the interspecific relationship is gene homology within the cereals and results presented suggests a tendency towards greater diversity in gene products (enzymes) as the seeds germinate. Such differences may be due to variation in architectural designs of the seed, with particular reference to organelles responsible for the production of these enzymes. Nevertheless, from a general outlook the interspecific relationship analysis showed that sorghum and wheat are more closely related to barley than rice, maize or wheat.

The malting process promotes solubilization of starch and proteins hence digestibility and nutritive value of the grains. The action of proteolytic enzymes are important during malting because they release amino acids and peptides required to buffer the wort, maintain yeast growth and also facilitate the breakdown of polysaccharide protein complex during mashing. Proteins in the endosperm are strongly associated with starch granules and they exhibit different levels of compaction in cereals. It is believed that the extent of solubilization of proteins is determined by the compactness of the polysaccharide, protein complex structure rather than the enzyme levels [17]. A given cereal may be resistant to endogenous protease enzymes, but readily susceptible to an exogenous source of the enzymes. Therefore it could be conceived that a mixture of the cereals would produce mixtures of endo-and exogenous enzymes that are capable of protein solubilization of the mixed malt, such a combination of cereals, might give good malt for food applications.

The use of blends of barley and other cereals otherwise called composite flours is not a new idea [18]. Blends of maize and sorghum meals are commonly used in many African countries. The need for the standardization of grains which may be used for composite flours has been highlighted. Biochemical criteria may be useful in this regard.

4. Conclusion

Based on this study, it is therefore suggested that a combined enzyme analysis will be useful. Such enzyme tests will predict the acceptability and digestibility of the mixed meals, and this will also facilitate decision-making criteria for the choice of the composite flour.

Table 1. Amylase lipase and protease levels in untreated, 24 steeped and malted samples and mean deviation

Samples & treatment	Amylase(units/mg protein)	Lipase(units/mg protein)	Protease(units/mg protein)
Barley			
Untreated	7.3 + 1.06*	100.0 +	256.7 + 11.5
24h Steeped	6.0+ 0.06	113. 3 + 5.8	186.7 + 11.5
48h sprout	118.8 + 5.3 ^b	186.7 + 7.6 ^a	313.3 + 11.5 ^a
Maize			
Untreated	4.0 + 0.5	73.3 + 5.8	2067 + 5.8
24 steeped	2.7 + 0.3	86.7 + 5. 8	180.0+ 20.0
48h sprout	35.3 + 1.2 ^a	256.7 + 15.3 ^b	303.3 + 11.5 ^a
Millet			
Untreated	2.2 + 0.3	86.7 + 5.8	296.7 + 5.8
24h steeped	5.3 + 1.0	200.0 + 10.0	256.7+ 32.1
48h sprout	31.2 + 0.3 ^a	233.3 + 11.5 ^b	293.3 + 5.8 ^b
Rice			
Untreated	2.2 + 0.3	86.7 + 5.8	296.7 + 5.8
24 steeped	2.3 + 0.3	103.3 + 5.8	3.36. 7 + 11.5
48 sprout	40.7 + 3.1 ^a	150.7 + 5.1 ^a	373. 3 + 11.5 ^b
Sorghum			
Untreated	4.0 + 0.12	73.3 + 11.5	186.3 + 5.8
24h steeped	5.3 + 0.3	83.3 + 5.8	183.3 + 5.8
48h sprout	32.0 + 2.0 ^a	188.3 + 2.9 ^a	303.3 + 5.8 ^a
Wheat			
Untreated	2.0 + 0.1	80.0 + 10.0	333.3 + 15.3
24 steeped	23.3 + 3.1	106.7 + 5.8	263.3 + 5.8
48h sprouted	52.3 + 2.3 ^b	195.0 + 5.0 ^a	603.7 + 11.5 ^b

* All values are Means and Standard Deviation of three Determinations. Values with different superscripts (a,b) vertically differs significantly (p<0.05)

Table 2. Percentage similarities between barley and other cereals 48h sprouted based on the three enzymes

Treatment	Barley and other cereals	Percentage (100 %)
48h sprouted	Barley and maize	73.7
48 sprouted	Barley and millet	75.7
48 sprouted	Barley and rice	71.91
48 sprouted	Barley and sorghum	84.21
48 sprouted	Barley and wheat	41.11

Percentage similarities were calculated from the mean deviation of the values obtained of the other cereals to barley as described in the text.

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